EFFECT OF DRYING METHODS AND EXTRACTION SOLVENT ON THE TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF PULP AND PEEL EXTRACTS OF *Benincasa Hispida*

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ABSTRACT

Benincasa hispida (*B. hispida*) also known as kundur, a member of cucurbitacea (cucurbit) family that gain highly attention as their biological function such as antioxidant, antimutagenic activities and high in polyphenol content. The foods that we eat contain high chemical composition especially ready to eat food thus, it is important to know the basic nutrition content from the food. With increasing the variety of food production, the increasing in antioxidant activity needed in order to prevent serious health's problem. Natural antioxidant usually comes from plant and from variety part of plants, it also contains its antioxidant value and phenolic content. The objective of this study is to evaluate how drying process of peel and pulp of *B. hispida* also by using different solvent can affect the antioxidant activity and total phenolic content (TPC) of the peel and pulp extracts. The effects of different drying proces (microwave dried and oven dried) and different solvent systems (ethanol, methanol, ethanol-water 80:20 and methanol-water 80:20) were assessed on the antioxidant activity and total phenolic contents of *B. hispida* peel and pulp. Antioxidant activities of the sample were determined through DPPH radical scavenging activity, while the TPC was determined spectrophotometrically using Folin-Ciocalteae assay. There was a difference in the extracting ability of each of the solvents. The aqueous solvents were superior in their ability to extract the antioxidants and aqueous methanol was significantly more efficient than aqueous ethanol as shown by the TPC results. As for DPPH, oven-dried pulp samples extracted by methanol solvent showed the highest scavenging activity at 96.55%. The pulp samples showed the highest radical scavenging activity of 81.98% (microwave-dried) and 97.80% (oven-dried) when extracted using 100% ethanol. Meanwhile the peel samples demonstrated highest radical scavenging activities at 68.35% (microwave-dried) and 81.84% (oven-dried) when extracted by aqueous methanol. The findings of this study revealed that 80% methanol and 100% ethanol are the best two extraction solvents used for obtaining the highest antioxidant activities Also, the peel and pulp samples drying process prior to extraction, also influenced the extraction yield. Oven dried peel samples had the highest yield while oven dried pulp had the lowest. From the result it shows that oven-dried has the best drying method by using aqueous methanol for antioxidant activity. While, for total phenolic content aqueous methanol show the best extraction solvent with microwave-dried. The result obtained demonstrated the potential of the peel and pulp of *B. hispida* as an alternative source of antioxidant agents.

ABSTRAK

Benincasa hispida (*B. hispida*) juga dikenali sebagai Kundur , ahli cucurbitacea (labu) kumpulan yang mendapat perhatian yang tinggi kerana fungsi biologikalnya seperti antioksidan, antimutagenic aktiviti dan tinggi kandungan polifenol. Makanan yang biasanya dimakan mengandungi komposisi kimia yang tinggi terutamanya makanan yang segera, Oleh itu adalah penting untuk mengetahui kandungan pemakanan asas dari makanan. Dengan meningkatnya pelbagai pengeluaran produk makanan, maka semakin meningkat aktiviti antioksidan yang diperlukan untuk mengelakkan diri daripada mendapat masalah kesihatan yang serius ini . Antioksidan semula jadi biasanya berasal dari tumbuhan dan dari pelbagai bahagian tumbuhan, ia juga mengandungi nilai antioksidan tersendiri dan kandungan fenolik. Objektif kajian ini adalah untuk menilai bagaimana proses pengeringan kulit dan isi *B. hispida* dan juga dengan menggunakan pelarut yang berbeza boleh menjejaskan aktiviti antioksidan dan kandungan jumlah fenol daripada ekstrak kulit dan isi. Kesan dari perberbezaan proses pengeringan (gelombang mikro dan ketuhar kering) dan perbezaan sistem pelarut (etanol, metanol , etanol - air 80:20 dan metanol - air 80:20) telah dinilai berdasarkan aktiviti antioksidan dan jumlah kandungan fenolik dari kulit dan isi *B. hispida*. Aktiviti antioksidan sampel ditentukan melalui aktiviti memerangkap DPPH radikal, manakala TPC telah ditentukan spektrofotometrikal menggunakan Folin - Ciocalteae assay. Terdapat perbezaan dalam keupayaan mengekstrak bagi setiap jenis pelarut. Pelarut yang mengandungi kandungan air mempunyai keupayaan yang lebih untuk mendapatkan antioksidan dan campuran metanol dan air adalah jauh lebih cekap berbanding campuran etanol dan air seperti yang ditunjukkan oleh keputusan TPC. Bagi DPPH, sampel isi dari ketuhar -kering yang diekstrak dengan pelarut methanol menunjukkan aktiviti memerangkap tertinggi pada 96.55%. Sampel isi menunjukkan aktiviti mengaut radikal tertinggi 81.98% (gelombang mikro-kering) dan 97.80% (ketuhar-kering) apabila diekstrak dengan menggunakan 100 % pelarut etanol. Sementara itu, sampel kulit menunjukkan aktiviti memerangkap radikal tertinggi iaitu 68.35 % (gelombang mikro-kering) dan 81.84% (ketuhar-kering) apabila diekstrak dengan campuran pelarut metanol dan air. Hasil kajian ini menunjukkan bahawa 80% metanol dan 100% etanol adalah dua pelarut pengekstrakan terbaik yang digunakan untuk mendapatkan aktiviti antioksidan yang tertinggi. Juga, proses pengeringan sampel kulit dan isi sebelum proses pengekstrakan juga mempengaruhi hasil pengekstrakan. Sampel kulit dari proses ketuhar kering mempunyai hasil tertinggi manakala sampel isi dari proses ketuhar kering mempunyai hasil terendah. Dari hasil kajian dijalankan ia menunjukkan bahawa ketuhar-kering mempunyai kaedah pengeringan yang terbaik dengan menggunakan metanol akueus untuk aktiviti antioksidan. Walaubagaimanapun, bagi kandungan jumlah fenol metanol akueus menunjukkan pengekstrakan pelarut terbaik dengan gelombang-kering. Keputusan yang diperolehi menunjukkan kulit dan pulpa *B. hispida* mempunyai potensi sebagai sumber alternatif agen antioksidan.

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LIST OF SYMBOLS

°C	Degree Celsius
%	Percentage
g	Gram
mg	Milligram
EC_{50}	Concentration of a compound decreasing the absorbance of a DPPH solution by 50 %)
M	Concentration
V	Volume
ml	Milliliter
nm	Nanometer
w/v	Weight per volume

LIST OF ABBREVIATIONS

DPPH	2,2-Diphenyl-1-picrylhydrazyl hydrate
TPC	Total phenolic content
FCR	Folin-Ciocalteu reagent
GAE	Gallic acid equivalent
OD	Optical density
ppm	Part per milliom
BHT	Butylated hydroxytouluene
UV	Ultraviolet
FRAP	Ferric-reducing antioxidant power
PG	Propyl gallate
TBHQ	Tert-butylhydro quinone
DNA	Deoxyribonucleic acid
ASAE	American Society of Agricultural Engineers

1 INTRODUCTION

1.1 Motivation and statement of problem

Antioxidants have been considered the medicine properties because of its potential to protect our body from the reactive oxygen species, reactive nitrogen species and reactive chlorine species (Shahidi, 1997). Antioxidants are the substance that helps to prevent deterioration that caused from oxidation such as loss of nutrient content by protecting the food we eat against it. Natural and synthetic compound contain its own antioxidant characteristic, only few of this characteristic can be accepted and categorized as safe for the food products by international bodies such as Food Additivies (JECFA) (Jan *et al*, 2001). At present, many antioxidants were produced synthetically, however, these synthetic antioxidant can inhibit the cancer activity, which is why more natural antioxidant is focused in order to defend from mutagenesis and carcinogenesis (Reische *et. al*, 1998).

Most natural antioxidants are come from plants and fruits. Flavonoids, carotenoids, ascorbic acid and tocopherols are some of example of antioxidant produced by plants. Flavonoid and phenolic such as phenolic acids, lignas and linin can be found in leaves, flowering tissue and woody parts. Variety of gourds have been suggested to have possible beneficial effect on health for example bitter gourd (*Momordica charantia)* may avoid from carcinogenesis (Hui *et al*., 2004;Singh *et al*., 1998). A kundur or wax gourd fruit is known as *Benincasa hispida (B.hispida)* are from cucurbitacea (cucurbit) family that contain mostly genetically diverse group and it is frost sensitive and have ability to tolerate with drought condition (Whitaker and Bohn, 1950). One of special things about *B.hispida* is that even through a year and many months, it can be stored without having any damages happen (Morton, 1971). Kundur fruits is popular among crops because it contains and provide good natural sources such as natural sugars, minerals, vitamin and amino acid. It also valued because of its natural nutritional contain and medicinal properties like anti-diarrheal, anti-obesity and antioxidant (Mingyu *et al*., 1995). There are few factors must be considered that can influence the rate of extraction and quality of extracted bioactive phenolic compounds, such as type of extraction solvent, solvent concentration, temperature and pH of extraction and extraction time (Chew *et al*., 2011 & Ng *et al*., 2012).

Extracting antioxidants from plant material most often involves the method of solvent extraction. The choice of solvent has been shown to have effect on the concentration of antioxidants extracted (Sultana *et al*., 2009; Ahmad *et al*., 2011). Study done by Durling *et al.* (2007) among aqueous solutions of ethanol use in a different concentration of 15% to 96% a better extraction yield of caffeic and rosmarinic acid were obtained with 30% and 60% ethanol solution. Little difference in extraction yield was found when ethanol, methanol, acetonitrile, acetone or water was used as extraction solvent. Hydroalcoholic mixtures of ethanol are possibly the most suitable solvent system for the extraction of sage polyphenols due to the different polarities of the bioactive constituents, and the acceptability of this solvent system for human consumption. The influence of different solvents like ethanol, methanol, acetone, acetonitrile and water on the proportion of phenolic acids as well as rosmarinic acid and caffeic acid in aromatic plants was done as water was apply as extraction solvent, 20% lower value of rosmarinic acid was obtained compared to other solvents (Wang *et al.,* 2004).

Antioxidant activity and extraction yields of antioxidant have been shown to be influenced by the drying procedure prior to extraction. Usually before extraction plant samples are milling, grinding and homogenization, it will process by air-drying or freeze-drying and generally, freeze-drying shows high levels of phenolics content in plant samples than air-drying (Abascal *et al.,* 2005). Study done by Asami *et al*. (2003) showed that freeze-dried Marion berries, strawberries and corn have a high total phenolic content level compared with air-dried Marion berries, strawberries and corn. However, drying processes, including freeze-drying, can cause effects towards the portion of plant samples, then, care step should be taken when running and evaluate the research studies on the medicinal properties of plants (Abascal *et al*., 2005).

Due to the study done there are not much research antioxidant of pulp and peel part. While much work has been conducted on the antioxidant content of *B.hispida,* there has been little work published on the effect of drying *B.hispida* prior to extraction or on the choice of extraction solvent. Thus this study presents the antioxidant activity and total phenolic content by using different drying method and different solvent of extraction. Antioxidant activity and extraction yields of antioxidant have been shown to be influenced by the drying procedure prior to extraction.

1.2 Objectives

The objectives of this research were:

- To compare the effects of oven drying and microwave drying on the total phenolic contents and antioxidant activities of B. hispida peel and pulp.
- To evaluate the total phenolic contents and antioxidant activity of B. hispida pulp and peel extraction of antioxidants using four different solvent systems (ethanol: water (80:20), 100% ethanol, methanol: water (80:20) and 100% methanol).

1.3 Scope of this research

In order to completing this research, a few scopes have been identified:

I. Pulp and peel of B. hispida was microwave dried for 25 minutes and oven dried at temperature 40°C for 3 days.
II. Extraction of antioxidants from the pulp and peel of B. hispida sample using ethanol:water (80:20), 100% ethanol, methanol:water (80:20) and 100% methanol.
III. Determination of the antioxidant activity using DPPH radical scavenging activity.
IV. Determination of the total phenolic contents (TPC) of each extract (pulp and peel) using the Folin-Ciocalteu's assay.

2 LITERATURE REVIEW

2.1 Introduction

Natural compounds can be the compounds that are suitable for research and design planning for a new discovery therapeutic development, biomimetic synthesis and new drugs (Hamburger and Hostettamann, 1991). The development of interest in the usage of alternative therapies and natural products of therapies specially that comes from plants because of it is harmless, effective and lack in side effects (Goldfrank *et al*., 1982;Vulto and Smet, 1988;Mnetz and Schenkel, 1989).

The exploration and acknowledgment of new and growth of the sources of functional food is because of the increasing demand from the customer for healthful foods. The fruits and vegetable contain the potential uses as the functional food ingredients that lead to the increasing interest among researcher to study through the current year. Many agree that consumption fruits and vegetable is correlated with reducing the risk of gradual deterioration of organ and cell diseases that come with aging such as cataract and immune disfunction (Ames *et al.*, 1993;Liu *et al*., 2008;Siddhraju and Becker, 2007).

The important of basic nutrients and also non-nutrients phytochemicals comes from natural plants and vegetable consumption has been widely introduced because of its important that related to health care and also can avoid from cancers and chronic disease (Steinmetz and Potter, 1996). Cucurbit family is one of the most genetically various group of food plants in plant kingdom and they are sickly drained soil, drought-tolerant and frost-sensitive (Whitaker and Bohn, 1950).

Some of the curcubit family members are pumpkin, cucumber, and gourd (Robinson and Dacker-Walters, 1999). *Benincasa hispida* (*B. hispida*) is one type of cucurbit family which contain high probable as a function for food production (Yadav and Sarma, 2005). Some research by epidemiologic stated that consumption food can lower the risk of human disease like cancer and inflammation because of it contain high amounts of antioxidant compounds and natural biological sources (Aruoma, 1998).

2.2 Wax Gourd (Benincasa Hispida)

Kundur or wax gourd is known as *Benincasa hispida* and from cucurbitacea (cucurbit) family that contains mostly genetically diverse group and it is frost sensitive and has ability to tolerate with drought condition (Whitaker and Bohn, 1950). One of special things about this *B. hispida* fruits is even through a year and many months, it can be stored without having any damages happens (Morton, 1971). Kundur fruits provide and contain good source such as natural sugars, minerals, vitamin and amino acid. It also valued because of its properties as medicine like anti-diarrheal, anti-obesity and antioxidant (Mingyu *et al*., 1995).

Walters (1998) state that in tropical Asia, India and China, the hereditary generation people extensively enriched this fruits since fifth century. *B. hispida* fruits "probably a native of Malaysia" and arises in wild Jawa. The seed of kundur fruits contain high oil that very useful and preferable for oil industrial application because of its properties such as odourless and good appearance and colour (Mariod *et al.*, 2009). Because of high amounts of oils which are polyunsaturated fatty acid, it is very advantages to prevent heart disease and cancer instead of have a favourable nutritional content (Yehuda *et al.*, 2005).

According to MacWillian (2005) for immature and mature fruits have high moisture contents like 93% harmful weight portion and develop to 96% when it is matured. Moreover, for pulp of kundur fruits the protein and ash amounts are between 0.3% to 0.5%. Natural sugars are produce from mature and immature pulp of kundur are glucose and fructose, it is reduced as the fruits matured while for organic acid present like malic acid and citric acid will show an increasing contents as it matured (Wills *et al.*, 1984). This fruits gain highly attention as their biological function such as antioxidant and antimutagenic activities and high in polyphenol content (Kono *et al.*, 1995;Azizah *et al*., 2007).

2.2.1. Nutritional and Phytochemicals Composition

To know the quality of a food, the nutritional data are important parameters like moisture, protein, carbohydrates and fiber. Table 2.1 shows the nutritional composition of immature and mature kundur fruit from different countries.

Table 2.1: Proximate composition of immature and mature Kundur (Benincasa hispida) fruit (g/100 g of edible portion)

Country	Immature fruit						Mature fruit					
	Moisture	Protein	Carbohydrate	Fibre	Fat	Ash	Moisture	Protein	Carbohydrate	Fibre	Fat	Ash
Australia	93.80	0.70	2.70	2.10	0.00	0.70	96.80	0.30	1.10	1.50	0.00	0.30
Florida	95.80	0.47	2.69	0.56	0.02	0.45	96.20	0.40	2.24	0.68	0.03	0.45
Malaysia	N.A	N.A	N.A	N.A	N.A	N.A	94.50	0.50	4.00	0.50	0.20	0.30
China	N.A	N.A	N.A	N.A	N.A	N.A	96.70	0.40	2.56	0.58	0.00	0.27
USDA	N.A	N.A	N.A	N.A	N.A	N.A	96.10	0.40	3.00	0.50	0.20	0.30
FAO	N.A	N.A	N.A	N.A	N.A	N.A	96.20	0.50	2.30	0.60	0.10	0.30

N.A : Data are not available from source

Reference: Zaini *et al.*,2010

From the Table 2.1, it shows fat is in low content about less than 0.3% of edible weight portion for all countries.

Table 2.2: Vitamins and minerals profile of mature Kundur (Benincasa hispida) fruit (mg/100 g of edible portion)

Country	Vitamins				Minerals			
	Vitamin C	Thiamin	Riboflavin	Niacin	Sodium (Na)	Potassium (K)	Calcium (Ca)	Iron (Fe)
Australia	27.00	0.02	0.05	0.40	1.00	77.00	5.00	0.30
Malaysia	68.00	0.02	0.031	0.20	2.00	131.00	11.00	0.20
China	1.35	N.A.	0.02	0.46	0.14	81.86	23.32	0.49
USDA	13.00	0.04	0.11	0.40	6.00	111.00	19.00	0.40
FAO	20.00	0.03	0.03	0.20	5.00	111.00	17.00	0.40

N.A. : data are not available from the sources

Reference: Zaini *et al.*, 2010

Table 2.2 shows the vitamin and minerals of mature kundur fruits from different sources. From the table Malaysia shows the highest vitamin c and riboflavin content of edible portion fruits compared to other country. From the table, it show potassium and calcium are major minerals content in kundur fruits. MacWillian (2005) stated that both potassium and calcium can give benefit as electrolytic balance of body fluid and alkalinizing the body. For amino acid content in different parts of mature kundur fruit has been studied by Mingyu *et al.* (1995) can be seen in Table 2.3. From table, it can be seen that total amount of protein and free amino acid high in skin part and free amino acid in the pulp part is the lowest while protein amino acid high in skin part and free and free amino acid high in seed part of kundur fruits. From this information the protein

and free amino acid, it can give potential source for dietary purpose. Besides that kundur fruits also are an important source of water (Mazumder *et al.*, 2005). From study done by Mazunder (2004), it shows that kundur fruits of insoluble residue contain high amount of homogalacturonan and D-galactan and a little acidic arabinan.

Table 2.3: Amino acid contents (mg/100 g fresh weight basis) in different parts of mature Kundur (*Benincasa hispida*) fruit

Amino acid	Protein amino acid			Free amino acid		
	Pulp	Seed	Skin	Pulp	Seed	Skin
Ornithine	7.002	6.946	N.A.	3.787	6.127	1.974
Aspartate	37.041	559.282	99.860	11.203	138.565	12.698
Threonine	7.325	171.905	34.078	20.889*	3.727	16.747
Serine	8.487	253.473	45.395		10.184	3.447
Glutamate	54.083	990.661	112.985	25.227	10.549	46.139
Proline	3.502	137.955	35.117	N.A.	N.A.	N.A.
Glycine	6.109	324.061	46.829	0.219	0.484	0.468
Alanine	8.507	244.525	54.288	1.623	3.047	12.056
Cysteine	1.505	40.186	2.755	0.715	3.513	1.013
Valine	7.128	200.942	39.933	1.087	3.673	2.448
Methionine	N.A.	30.883	3.711	0.410	2.161	0.501
Isoleucine	8.360	191.723	39.535	3.431	10.713	6.350
Leucine	9.548	348.316	62.567	0.714	3.841	1.794
Tyrosine	4.433	70.980	25.912	0.423	1.600	0.719
Phenylanine	8.221	267.765	46.566	4.072	8.823	4.867
Lysine	8.921	261.668	60.646	0.752	2.269	1.634
Histidine	6.009	133.558	24.170	1.134	3.497	2.539
Tryptophan	N.A.	N.A.	N.A.	1.079	2.919	2.017
Arginine	26.514	747.042	64.388	13.642	43.142	24.036
γ-Arminobutyric acid	3.673	9.869	N.A.	2.142	5.532	10.288
Total	216.400	5714.017	798.735	92.549	264.366	152.059

N.A.: data are not available from the source

* Total of theorinine and serine

Source: Zaini *et al.*, 2010

2.2.2. Health Benefits and Medical Properties

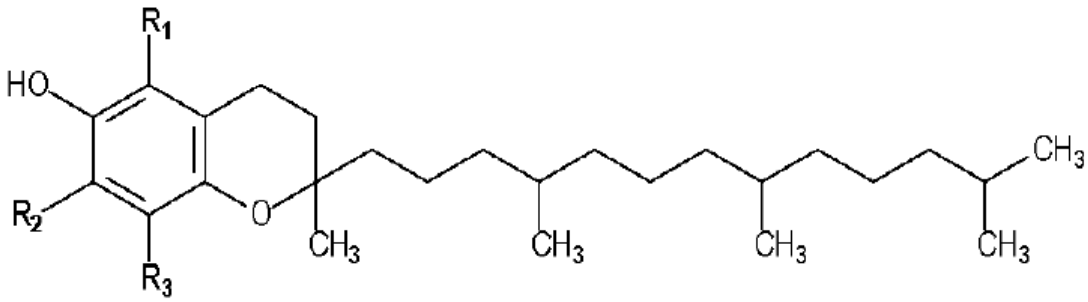
Plant is the species which commonly used for medication either by modern or traditional medicine system and most of this species would use it to cure and treat chronic health problems. Antioxidant properties contain in many extracts from plant besides minerals and primary metabolites (Akinmoladun *et al.*, 2007). Plants such as *Benincasa hispida* (*B. hispida*) have been used to cure the diabetes melitus, urinary infection and chronic inflammatory disorder (Grover and Rathi, 1994;Lee *et. al.*, 2005). For the juice from the kundur fruits extract shows that it can be anti-ulcer, diuretic activities and anti-depressant (Mingyu *et al.*, 1995). The juice of kundur fruits extract has antioxidant activity according to the study by Huang *et al.* (2004) and Roy *et al.* (2007). Kundur fruits have potent antioxidant activity on the kidney and were studied on albino rat, according to the result, kundur fruits can decrease the renal damage that is due to the radical scavenging activity (Bhalodia *et al.*, 2009). Kundur fruits also can protect and prevent the kidney injury that is done by mercury chloride (Mingyu *et al.*, 1995). There is also some study found that the seed of kundur fruit have possibilities to be angiogenic inhibitor that is to prevent the tumor growth and obesity (Lee *et al.*, 2005).

2.3 Phenol Component

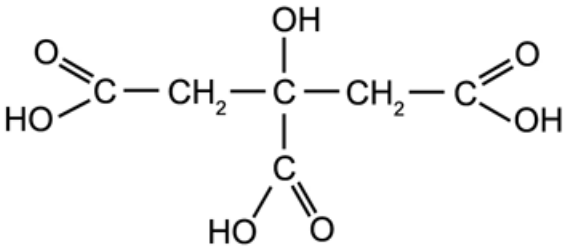
Phenolic compounds contain in various vegetable foods such as fruits and nuts and suggested that it can give good antioxidant effects. Besides that essential oil and various plants extracts has gain interest because of its potential as best antioxidant properties for food preservation (Zygadlo *et al.*, 1995;Maestri *et al.*, 1996;Maestri *et al.*, 1997;Tepe *et al.*, 2004). Phenolic is a substance that contain one or more hydroxyl group (OH) substituents bonded to an aromatic ring and because of its chemical structure, it have ability to delocalize phenoxide ion that can lose a further election to form corresponding radical which is also can delocalize (Waterman and Mole, 1994).

Phenolic are heterogeneous groups of secondary plant metabolites, they have involved in UV protection, nodule production and pigmentation. It has several of structure. The main phenolic compounds are flavonoids, tannins and phenolic acids (Waterman and Mole, 1994;Koes *et al.*, 1994;Burns *et al.*, 2001;Rababah, Ereifej and Howard, 2005). However, the uses of phenolic in food are limited by its requirement in order to make

food is safe to eat. The monohydric or polyhydric phenols are the main lipid-soluble antioxidant that is used in food with variety of ring substitutions. The combination of primary antioxidant of phenolic antioxidant with variety metal sequestering agents such as tocopherols with citric acid (Figure 2.1) and isopropyl citrate is used for maximum efficiency (Nawar, 1985).



Tocopherol structure

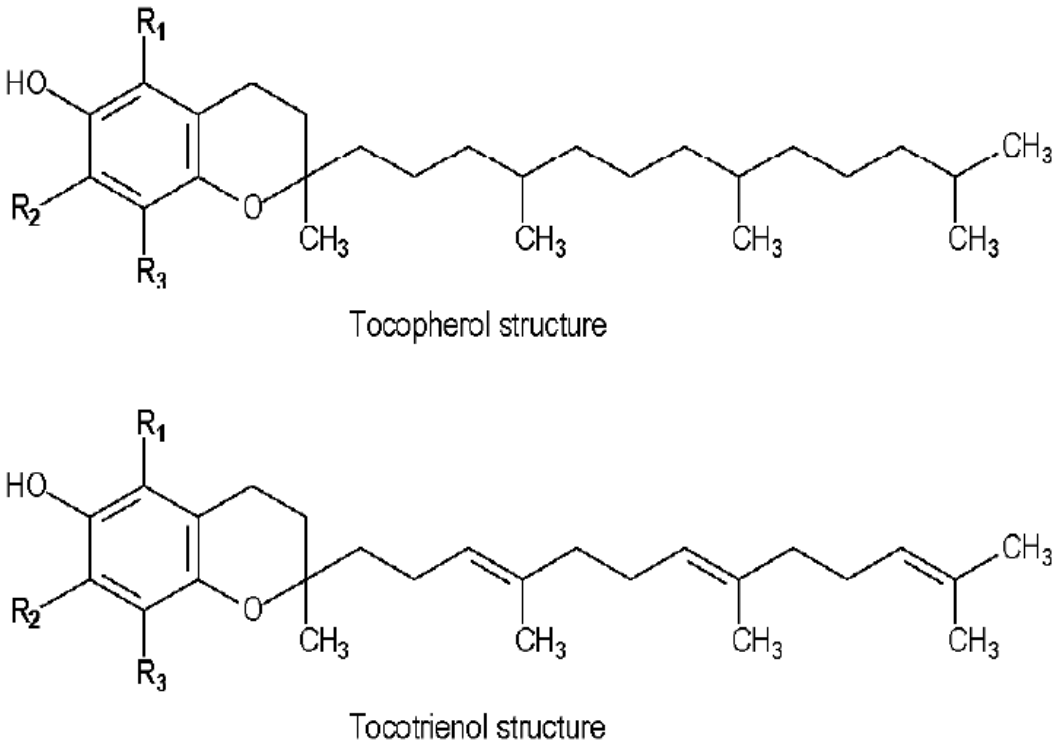


The citric acid molecule.

Reference: Zaini *et al.*, 2010

Figure 2.1: Tocopherol and citric acid structure

Natural phenolic compounds can be categorized as lipophilic group (tocopherols) and hydrophilic group (phenolic acids and flavonoids) which contain antioxidant properties. For lipid-soluble antioxidant that present naturally in vegetable oils, the compounds is tocopherols and tocotrienols as both have same ring structure that shown in Figure 2.2 but tocotrienols contain unsaturated carbon chains (Hashim *et al.*, 1993;Shintani and Della, 1998;Holownia *et al*., 2001).



Source: Zaini *et al.,* 2010

Figure 2.2: Tocopherol and tocotrienol structure

Phenolics acids are the another groups of phenolic compounds which is contain antioxidant properties such as gallic acic are used as starting compound to form food additives (Kubo, 1999;Hynes and Coincenainm, 2001;Aruma *et al.*, 1993).

2.4 Antioxidant

Antioxidant is the substances that help to prevent deterioration that caused from oxidation such as loss of nutrient content by protecting the food that we eat against it. Natural and synthetic compound contain its own antioxidant characteristic, only few of this characteristic can be accepted and categorized as a safe characteristic to introduce for the food products by international bodies such as Food Additives (JECFA). This antioxidant have been consider the medicine properties because of its potential to protect the body caused by the reactive oxygen species, reactive nitrogen species and reactive chlorine species (Shahidi, 1997;Freidoon and Ying, 2005). Antioxidant is dividing into some classes due to its actions mechanism. It can be categorize as primary and secondary antioxidant. For primary antioxidant it break the chain reaction of antioxidant by donate the hydrogen molecule while for secondary antioxidant it react by

slower the oxidation rate by a various reaction such as scavenging of oxygen, inactivation of hydroperoxide (Freidoon and Ying, 2005).

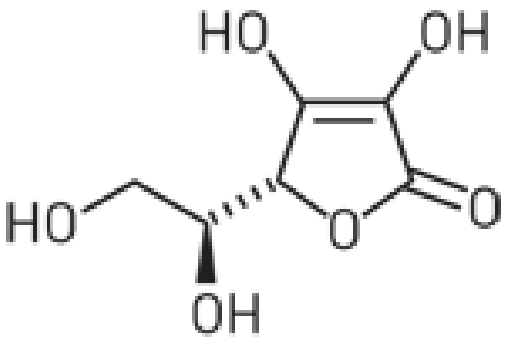
2.4.1 Impact of Antioxidants on Health

Antioxidant have potential to reduce oxidative loss together with loss from lipid peroxidation and it can block several disease like atherosclerosis aging and inflammation (Huang *et al.*, 2004;Roy, Ghosh and Guha, 2007). Antioxidant was discovered by M. Van *et al*. (2004) that it can block lipids against oxidation by destroy free radicals or scavenging oxygen among others. In food, the low concentration of antioxidant compared to oxidizable compound can lower and block the oxidation of substrate (Shahidi, 2000). Antioxidants are used in health area because of its potential to block the damage done by reactive oxygen species and reactive nitrogen species also reactive chlorine species towards the body (Shahidi, 1997).

There is a lot of reason our body will produce reactive species than we need it includes too much fat, alcohol, smoking and even too much exercise. One of the substances that can cover and reduce this reactive species is antioxidants. Reactive oxygen species and reactive nitrogen species are high in our body, it can deactivate oxidize lipids enzyme and cause our genetic materials damage (Mbata, 2005). In human body, it contain complicated and derived from deeply degrade antioxidant protection system. It contains many types of components such as endogenous and exogeneous connection that can interactively and synergistically in order to clean the free radicals. The component involves are nutrient-derived antioxidant (example: vitamin C), antioxidant enzymes (example: gluthathione peroxidase), metal binding proteins (example: albumin) and other nutrients that come from many types of plants

2.4.2 Natural and Synthetic Antioxidant

Commercially the example for natural antioxidant are tocopherols (vitamin E), ascorbic acid (vitamin C) (Figure 2.3) and rosemary extract (Valenzuela, Sanhueza and Nieto, 2000;Löliger, 1991).



Vitamin C

Source: Zaini *et al.*, 2010

Figure 2.3: Structure of vitamin C

Not all synthethic antioxidant are usually used in food, the only synthethic antioxidant used are butylated hydroxyanisole (BHA), butylated hydroxytouluene (BHT), propyl gallate (PG) and tert-butylhydro quinone (TBHQ) (Shahidi, 2000;D.F. and M.K., 1997). Synthetic antioxidant that is used in food industry is usually added as direct additives or indirect additives through packaging material diffused (M. Van, 2004). This is because all antioxidant have advantage and disadvantage, so that such thermal stability, effective concentration and synergism must be take note when select the antioxidant to used in several food. Because some of antioxidant has show potential that affects our health, the regulatory status of antioxidant must be considered. Tested for the synthetic antioxidant in safety to use in food are approve and used in low concentration on basis complex toxicity (Reische *et al.*, 1998).

2.5 Extraction Process

Extraction is the process of the partitioning of a solute between two immiscible or partially miscible phases. Liquid-liquid extraction happen when extraction takes place from one liquid to another liquid and solid-liquid extraction or leaching happen between liquid and solid, where the liquid is used to extract solutes from solid substance. This extraction process is using in some bioprocessing process such as purification of antiobiotics, purification of DNA, purification of lipids and others (Raja Ghosh, 2006).

2.5.1 Factors Affecting Extraction Process

In the extraction process some factor must be considered in order to get high efficiency result. Some of the factor that affects extraction process is drying time, solvent polarity and solvent to solid ratio. When the extraction involve one liquid medium to another liquid medium is called as liquid-liquid extraction while when solid medium to extract liquid medium involve it is refer as leaching (Raja Ghosh, 2006). Factors affecting extraction are temperature, pressure and flow-rate. The properties of the matrix and the analytes also affect the extraction. The selectivity of the extraction can be tuned by change in temperature and pressure and by the choice of an appropriate trapping method for the analytes (Miller and Hawthorne, 1998). The advantages of solvent extraction over other methods of oil expression include, higher oil yield (about 95% of the oil content could be obtained), larger processing capacity, solvent extraction also gave oil that many considered to be of superior bleaching quality, lower refining losses, reduced susceptibility to rancidity and better retention of fat - soluble vitamin, (Robbellen *et al*., 1989).

2.5.2 Extraction Solvent

Solvents that is used in the food must be appropriate in order not to make it harmful towards us. The solvent are allowed like water (with admixture of acid or base), other foodstuff with solvent properties and solvents like propene, butane, ethyl acetate, ethanol, CO2, N2O, and acetone (the latter not with olive oil) are allowed according to European Union and governmental regulations. In Table 2.4 and Table 2.5 shows that the solvents with food stuffs and maximal residue content also the residue in artificial flavoured products.

Table 2.4: Solvents with foodstuffs and maximal residue content

Solvent	Purpose	Maximum residue
Hexane	Fractionating of fats, oils or cacao butter	1 mg kg^{-1} in oil, fat or cacao butter
	Defatting of protein containing products respectively flour	30 mg kg^{-1} in defatted soy products, otherwise 10 mg kg^{-1}
	Defatting of corn seed	5 mg kg^{-1} in defatted seed
Methlyacetate	Extraction of for example, caffeine or other bitter constituents from tea or coffee	20 mg kg^{-1} in coffee or tea
	Production of sugar from molasses	1 mg kg^{-1} sugar
Ethylmethylketone	Fractionating of oils and fats	5 mg kg^{-1} in oil or fat
	Extraction of for example, caffeine or other bitter constituents from tea or coffee	20 mg kg^{-1} in tea or coffee
Dichloromethane	Extraction of for example caffeine or other bitter constituents from tea and coffee	2 mg kg^{-1} in roasted coffee and 5 mg kg^{-1} in tea
Methanol	For all products	10 mg kg^{-1}
Propane-2-ol	For all products	10 mg kg^{-1}

Reference: Hans-Jörg Bart and Stephen Pilz, 2011

Table 2.5: Residue in artificial flavoured products

Solvent	Maximum residue (mg kg^{-1})
Diethylether	2
Hexane	1
Cyclohexane	1
Methylacetate	1
Butane-1-ol	1
Butane-2-ol	1
Ethylmethylketone	1
Dichloromethane	0.02
Propane-1-ol	1
1,1,1,2-Tetrafluoreoethane	0.02

Reference: Hans-Jörg Bart and Stephen Pilz, 2011

The selectivity of solvents depends on selectivity, recoverability of solvent, viscosity and melting point, surface tension, toxicity and flammability, corrosively, thermal and chemical stability, availability and cost and lastly depends on environmental impact. Fresh plant material with organic solvents such as ethanol and methanol is preferable because denaturing of enzyme and conserve the solute undamaged (Eggers and Jaeger, 2003).

2.6 Drying Method

Besides extraction yields of antioxidant, the drying effects also can influence the antioxidant activity, this is based on studied by Chan *et al*. (2008), the thermal drying methods tested like microwave dried, sun dried and oven dried shows the decreasing in total phenolic content of leaves and tea ginger. A study by Mrkic *et al*. (2006) shows that the drying time is the main factor of antioxidant activity, when use shorter drying time in high temperature and increased air flow the maximum antioxidant activity is produced. So, in order to detect the natural antioxidant besides we focus on plants that high in oxidant activity, the extraction and drying factor must be consider. In the step preceding drying process, usually the final or desired products are in an aqueous solution and final level of purity. Drying method also necessary used to remove unwanted volatile substance. For all the material water contained inside the solid material in two forms that is unbound or free water and bound water. Unbound or free water is free to equilibrium with water which is in vapour phase and has the same vapour pressure as bulk water. While for the bound water can exits in several condition which is water in fine capillaries that have low pressure because of high concave curvature of the surface. Second condition when water has high level of dissolved solid and lastly when water in physical or chemical combination with solid (Roger *et al.*, 2003).

2.6.1 Principle of Oven Drying

Oven drying is harder to control than drying with a dehydrator but some products can be quite successfully dried in the oven. It usually takes two to three times longer to dry food in an oven. Compared to the other methods, oven drying methods are the simplest. There are two kinds of drying ovens which is hot air ovens and vacuum ovens. Air

ovens are more comfortable and cheaper than vacuum ovens. An air oven method was taken for the ASAE standards (ASAE, 1982). Air drying saves energy costs and reduces required dry furnace amounts. Limitations of air drying are generally involved with uncontrolled drying. If air circulation is too slow, a longer time is needed for the surfaces of the material to grasp moisture equilibrium. Warm, humid periods with little air movement may boost the growth of fungal stains, as well as aggravate chemical stains (William, 1999). Hart *et al*. (1959) show that drying flaxseed that have moisture content of 7.6 to 8.20 wet basis at temperature100℃ to 130℃ , wheat 12.0% to 12.3% at temperature 100℃ to 110℃ and corn 10.5% to 11.4% at temperature 94℃ to 105℃ until it achieve constant weight. It shows small different moisture content. It can increase the differences of moisture content by using wide ranges of grain moisture content. Bowden (1984) compared three types oven method for wheat and barley in determination on moisture content. For both study it can conclude that many types of moisture content within the replicates will increased the level of moisture content. Study done by Ayodele *et al.* (2011) show that mushroom with sun drying can maintain high nutrients and minerals compared to oven drying and smoke drying. As conclusion oven drying is not the best type of drying method in order to maintain minerals and nutrients. Also for moisture content oven drying can give low attained moisture content that can be affected by the length time of drying.

2.6.2 Principle of Microwave Drying

Over the years there has been an increasing interest in microwave drying in order to reduce drying time and increase the removal of water from agricultural products. Microwave drying has several advantages such as high in drying rate, short in drying time, decrease in energy consumption, and good result of the dried products (Sanga *et al*., 2000). Based on the fast drying time of microwave heating, microwave-convective drying of fruit has shown success in obtaining high quality dried product with low specific energy consumption (Tulasidas *et al*., 1997; Raghavan and Silveira, 2001). One of the main advantages of using the microwave heating is that the temperature and moisture gradients are parallel in direction, and help each other as opposed to conventional heating where moisture must move out from the material against the different of temperature (Murthy and Prasad, 2005). Tulasidas *et al.* (1995) was studied about the drying of grapes by using microwave dried, the factor that consider include